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for the development of

Biosatellite Project Experiment P-1096

"Emergence of Seedlings under Zero Gravity"

conducted with other experiments under the title

THE EFFECT OF WEIGHTLESSNESS ON THE GROWTH AND ORIENTATION OF ROOTS AND SHOOTS OF MONOCOTYLEDONOUS SEEDLINGS

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I. INTRODUCTION

1. OBJECTIVES

The successful development of the first phase of our biosatellite experiment with seed germination and seedling growth has required the design, assembly and extensive testing of a practical system for growing healthy seedlings in a small, sealed container that can be accommodated in the spacecraft and recovered without damage to the plants after a 3-day orbit. In order to minimize the possible interference effects of launch vibration and acceleration on later growth of the embryo, it has also been necessary to plan on pre-soaking of the dormant seeds only just far enough in advance of launch to permit identification and selection of partly swollen grains with vigorous embryos. In so far as possible all growth processes are to be carried out with the force of gravity at or very close to zero.

Another objective in developing a system for growing seedlings under the essentially weightless conditions of orbital flight was to provide for return of the plants with maximum amounts of tissues for biochemical analyses by one team of experimenters (Drs. Conrad and Johnson of North American Aviation) and for anatomical and histochemical studies by a second team of experimenters (Drs. Edwards and Gray of Emory University) without compromising the requirements for our analysis of the orientation of the seedling organs in the absence of the gravitational force to which such seedlings have become adapted during the evolution of the seed plants. This study of gross morphology as affected by the elimination of the force of gravity imposed a serious constraint on the method of growing the seedlings, since the contact of their roots with any solid particles could not be permitted.

2. WHEAT SEEDLINGS

The choice of wheat as the test plant was determined by factors of size, rapidity and reliability of germination, and previous work with wheat seedlings subjected to strong centrifugal forces (3,5). Seeds for all tests of experimental systems (and later for the orbital experiment) are being taken from a certified stock of _Triticum aestivum_ L. (_T. vulgare_ Vill.) hort. var. Georgia 1123 (4). Supplies of the latest crop are obtained in late summer from disease-free stocks with very high percentages of germination.

The special value of wheat seedlings for experimental study of the orientation of coleoptile and roots in the absence of tropistic effects of gravity has been shown (see p. 29) by comparative tests of wheat, rye, barley and oats. When grown in the special seed holders designed for our experiment (see Figs. 1 and 3 and text below), the regularity of the root system in wheat provides for quantitative work that could not be carried out with any other cereal grain. Wheat alone has a pattern of two lateral (seminal) roots which grow at characteristic, predictable angles to the primary root, both in our seed holders and when grown in soil (cf. Table V, p. 31).

3. CULTURE METHOD

In order to grow the wheat seedlings without contacts between their roots and solid particles that would introduce the effects of thigmotropism or traumatropism, it was necessary to devise a method that would allow the roots to grow in all possible directions from the embryo without touching each other or any other objects. The orientation of roots is fully as important as that of the coleoptile in a study of geotropism.

A relative humidity of 100%, corresponding to the conditions found within the pores of an arable soil, was also an essential requirement. Since the volume of water needed for growth of an embryo into a seedling can not be obtained by diffusion from the water vapor in moist air, the successful development of a large seedling during a 3-day experiment required delivery of the water to the embryo by way of the endosperm. This part of the seed was also the only available region for holding the seed and later the seedling while the young plant grew in moist air of normal composition for root environment and at a favorable temperature for rapid development.

These cultural requirements were met by an original method of suspending seeds in sets of 12 or 15 within a moist chamber, with part of each seed held firmly in contact with films of water. The design of this special seed holder was worked out jointly with the other experimenters with wheat seedlings. We had the technical assistance of engineers at the Ames Research Center and at the laboratories of North American Aviation where acceptable models were fabricated for test hardware.

II. DEVELOPMENT OF SEED HOLDERS

1. EARLY MODELS

Proof of the capacity of the wheat endosperm to absorb and transmit enough water for growth of the embryo was established first with a branching system of plastic tubes to which soaked seeds were attached by short, cloth wicks. Each wick was hollow, with a diameter just large enough to slip over a plastic side arm tube that was joined to the central reservoir. The endosperm portion of a soaked seed was inserted into the outer end of the wick and held against the plastic tube and its contents by means of a short, steel needle which passed through the wick and endosperm without contact with the embryo.

With the plastic tubes filled with a slurry of fine vermiculite and water, the cloth wicks carried the water to the seeds by capillarity. Each set of germinating seeds was suspended within a plastic cylinder to which was added enough water to maintain 100% relative humidity around the seedlings. Tests showed that the tubes supplied enough water for at least 6 days of germination. The moist air preserved the root tips and root hairs and thus provided for the growth of normal seedlings.

For practical reasons, the method was later changed to insertion of the endosperm directly into the tubular side arm, in contact with the wet, porous medium of the reservoir system. Trials of side arms with inner diameters scarcely larger than the diameter of a partly swollen seed (2) proved that growth ceased when the expanding seed filled the space and reduced the area of contact with the water. The minimum internal diameter of the seed holder arm was found to be 7/32 inch, a size that allowed for a film of water on all submerged surfaces of the endosperm region when it was fully expanded.

2. PREPROTOTYPE HOLDERS

The model selected for use in the preprototype hardware is a system of plastic tubes in which a central stalk has 12 or 15 side arms, each set at an angle of 45° with one end of the central reservoir (see Fig. 1). The short side tubes are arranged in 4 or 5 spiral sets of 3 each, with the vertical spacing determined by the lengths and forms of seedling organs when they were grown in earlier models that were being rotated on a horizontal clinostat (see p. 15). When the system is loaded for an experiment, the central tube is sealed at each end with an Allen screw after all air bubbles have been expelled.

The enlarged detail of one arm in Figure 1 shows how the seed is planted by inserting it through a small hole in a rubber membrane that forms a cap over the side arm. The thickness of this rubber dam, as used in our work, has been either 1/128 in. or 1/65 in., with equally good results in holding the seed and sealing the system. The rubber band shown in the figure can be either an O-ring or a tiny band of latex rubber, either of which forces the membrane into a groove in the side arm wall near the end of the arm.

Molded rubber caps have been tested and found to be applied more easily. They also offer less surface for unwanted contact with seedling organs and for screening views of organ tips in photographic records of growth. These caps are being designed by Dr. K. Yokoyama, our collaborator in the project at the Ames Research Center where they will be fabricated in quantity when an acceptable model has been developed there.

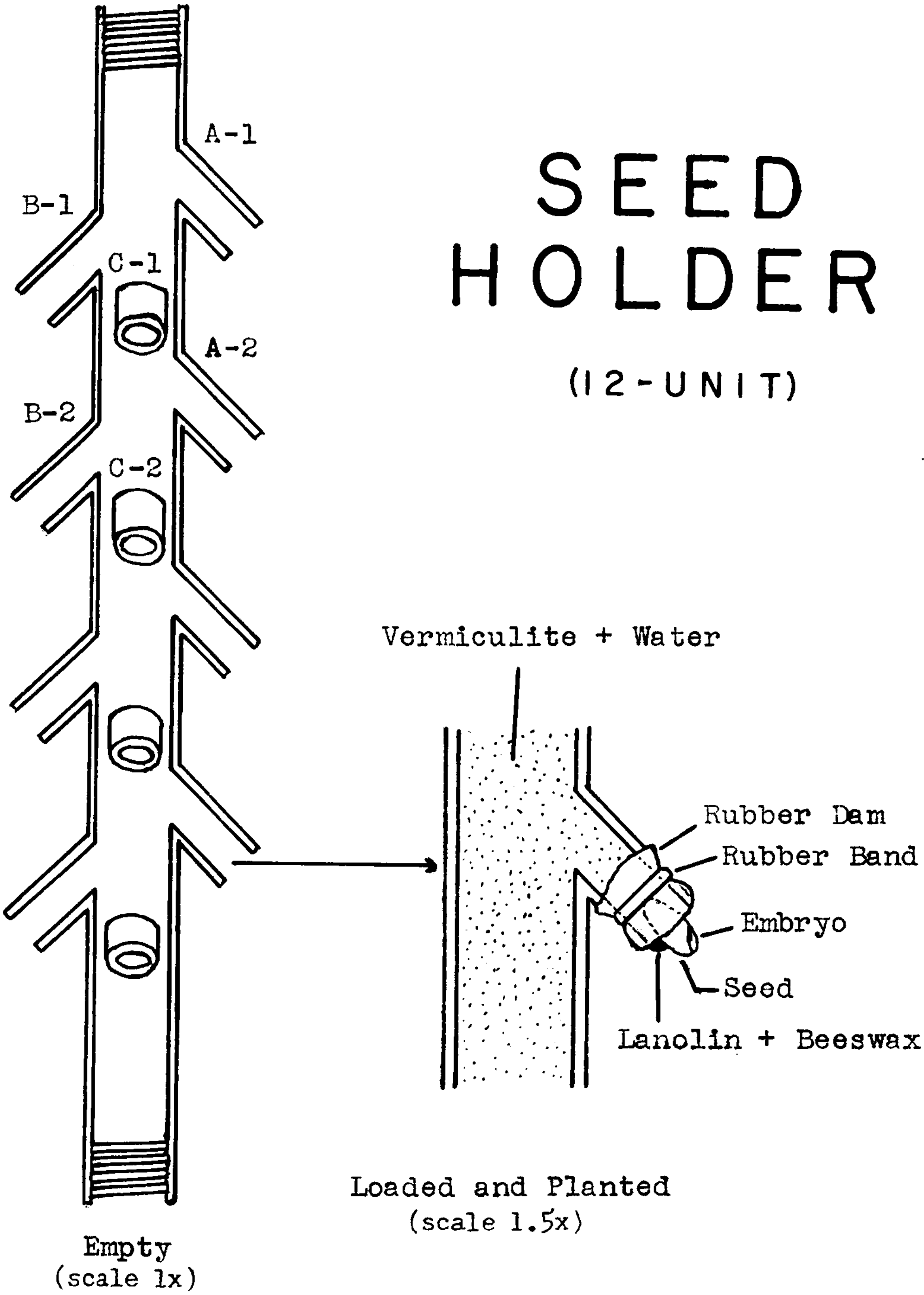

Fig. 1. Holder System for Preprototype Package.

In order to prevent leakage of water from the side arm by way of the groove in the seed on the side opposite the embryo, a blended mixture of lanolin and beeswax (2 to 1 by wt.) is applied after the seed has been planted as shown in the figure. A small drop of the mixture, just warm enough to liquify, is placed as indicated but with the loaded and planted system inverted for this operation. The mixture solidifies rapidly, adheres to the rubber and seed and thereby aids in holding each seed in place with proper orientation of the embryo as shown in the diagram.

During the final orbital experiment, this seal will also help to hold the seed during launch and the seedling during reentry. The rubber membrane, however, is very effective in gripping the seed while serving the even more important function of damping some of the vibration during launch. In tests thus far, no seed has been dislodged by the simulated profile of launch vibrations or by the drop test during simulated recovery stresses.

3. MATERIAL TO HOLD WATER AROUND THE SEED

The choice of medium to hold water inside the seed holders has proved to be an exacting step. The material must provide an evenly porous framework for capillary flow of water to each seedling during 3 days of germination. It is also important that the solid material in the side arm not shrink away from the seed coats as its water moves into the seed.

Particles of commercial "Vermiculite" that pass through a sieve with 20 mesh per inch proved effective for early tests but some lots were inferior. A series of tests with "Perlite", agar gels and powdered cellulose, alone or mixed with vermiculite, was carried out over a period of many months. Table I shows that cellulose alone was better than any other material tested in tubular holders during 1964.

Mixtures of cellulose and fine vermiculite proved equally effective in later tests (see Tables III and IV) but the best material of all seems to be a vermiculite powder that passes a 40-mesh sieve but is held in an 80-mesh sieve. The uniformity of the spaces between the particles also permits pre-loading of the seed holders before the water is added as described on p. 16. Results with this material and loading method are entered in Table IV where the medium is listed as very fine vermiculite.

Table I

Growth of Seedlings for 72 Hours with Different Media

1964	Seed Holder		Media	Seedling		Mean Lengths in mm.			
Date	Position	Type		Nos.		Coleop.	P.Root	L.Root	R.Root
Nov. 7	Erect	Old	Vermiculite	12		3.1	18.1	10.9	10.1
	Clino.	"	"	12		3.4	22.5	15.0	14.2
" 12	E	"	"	12		2.8	18.0	12.9	13.3
	C	"	"	12		2.8	21.3	8.3	10.8
Dec. 1	E	New	Perlite	15		2.6	13.1	7.6	8.1
	E	"	"	11		2.1	7.7	5.8	6.3
	C	"	"	13		3.4	21.0	14.2	14.2
" 4	E	Old	0.25% Agar	12		3.0	14.0	8.1	7.8
	E	"	"	11		3.1	13.0	9.1	10.4
	C	"	"	11		4.1	19.8	13.0	13.0
	C	"	"	11		2.6	15.4	11.6	10.6
" 7	E	New	0.5% Agar	15		4.7	21.5	18.6	14.7
	C	"	"	15		6.4	23.4	19.8	17.8
" 10	E	"	1% Agar	11		5.6	16.1	13.7	13.7
	C	"	"	15		3.3	17.0	12.0	12.4
" 11	E	"	"	12		2.5	18.3	11.3	10.4
	C	"	"	14		2.6	13.7	11.1	10.0
" 14	E	"	"	12		2.3	14.9	4.4	3.6
	C	"	"	15		2.9	20.9	10.3	10.3
" 17	E	Old	Vermiculite	10		3.4	18.4	13.2	12.8
	C	New	"	14		4.2	25.1	16.5	13.6
	C	"	Cellulose	9	*	16.3	18.9	20.2	19.8
	E	Old	"	11		6.5	19.0	13.8	12.5
	C	New	"	13		11.0	25.0	17.2	14.8
" 21	E	Old	"	10		7.1	16.6	18.2	15.2
	E	"	"	11		6.0	18.6	14.2	15.0
	E	"	"	12		7.0	20.5	17.5	15.2
	C	New	"	9	*	15.6	28.2	23.7	21.2
	C	"	"	14		11.8	28.0	19.9	19.2
	C	"	"	13		9.8	27.0	22.0	19.5
" 26	C	"	"	15		4.7	20.1	15.0	14.3
	C	"	"	15		6.5	19.3	15.0	15.2
	E	"	"	12		6.7	19.4	17.4	18.3
	E	Old	"	12		6.5	18.3	15.3	13.3
Jan. 1	E	New	"	11		9.5	23.7	19.6	18.6
	C	"	"	14		9.0	19.3	18.6	16.9
	C	"	"	14		10.9	20.6	19.6	19.2

III. PREPROTOTYPE PACKAGE

The package for the experiment has been designed to hold 6 sets of seedlings in 4 non-toxic, plastic chambers, each with a metallic base, tight cover and a heating blanket to maintain an internal temperature of approximately 77° F. The sealed and wrapped chambers are attached to a shock-mounted base as shown in Figure 2 with 1 cylinder exposed to show the seed holder in place and the space for growth of its 12 seedlings.

The large chamber in the diagram has space for 3 sets of 12 seedlings on holders so spaced that the plants will grow free of each other until recovery after 3 days. The 3 cylindrical chambers have 1 set of seeds each, with 1 set of 12 to grow until recovery and the other 2 sets to have 15 seedlings each.

These sets of 15 seedlings will be killed and fixed during orbit, one set at 48 hours after launch and the other after 60 hours of orbit. The fixation will be done by spray from 2 nozzles as shown in the base of the exposed cylinder (Fig. 2). Each nozzle will throw 25 ml. of fixative forced from a steel cylinder underneath by means of a gas-driven piston on command from a program in the spacecraft electrical system.

In addition to thermostat control of the temperature in the growth chambers, the actual temperature in each chamber will be recorded through its thermistor, an electronic package attached to the base plate, and a connector for interface with the recorder in the spacecraft. The resulting record of temperature profile during the course of the experiment will provide a basis for revision of base line growth control data if there are significant deviations from the 77° F. objective.

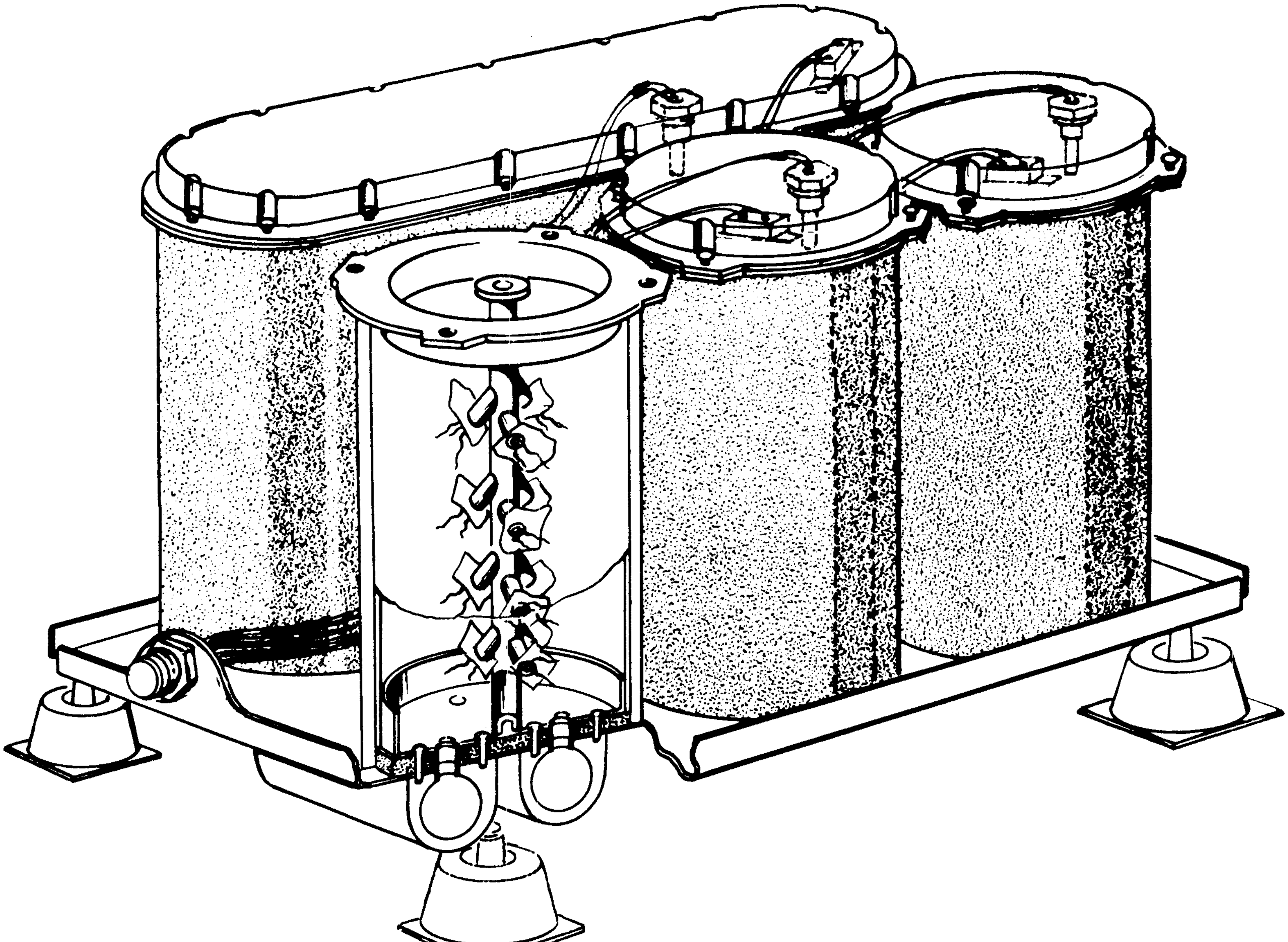

Figure 2. Perspective of Preprototype Hardware
(courtesy of No. Amer. Aviation)

IV. GROWTH TESTS IN PREPROTOTYPE HARDWARE

1. TESTS WITH INTACT PACKAGES

Development tests with the assembled packages and simulated stress profiles of launch and recovery were made at the Ames Research Center. The co-experimenters worked together and, with the assistance of personnel in the Biosatellite Project at ARC, planted the seeds in the holders, prepared the package as though for an orbital experiment, and observed the results after 3 days of growth between the applications of stress profiles by the staff at ARC. Control packages were prepared and used for parallel growth tests of seeds not subjected to the stresses of vibration and acceleration.

The test in November, 1964, failed to produce good seedlings in either control or experimental package, due to toxic effects of volatile, fabrication compounds. Even the largest seedlings in the chamber with 3 sets of unfixed plants were too small to evaluate the effect of the drop test on the security of the plants in the holders at re-entry time. The test did prove, however, that the combination of shock absorbers and rubber membranes around the seeds prevent dislodgement of any seeds during simulated launch.

The January, 1965, test with modified, non-toxic hardware was satisfactory for most of the biological phases of the experiment. The seedlings grew well in holders that had been properly loaded and planted. The system of culture was proved to be practical and all seeds and seedlings were retained during the stress profiles. The absence of geotropic stimulation during the 3-day orbit was simulated by turning the experimental package on a

2 rph horizontal clinostat, with temperature control and record as designed for actual orbit. The control package stood erect to gravity but otherwise had the same growth conditions although the seeds lacked possible effects of stresses during launch.

The measurements of the coleoptiles and roots produced by growth of the 6 sets in each package were reported in detail earlier (7). A summary of these data, together with measurements of angles used to describe the orientation of the seedling organs (see p. 19), is included in Table III on p. 26. The letter F after the hours of growth for the first 3 sets of seedlings in each package indicates fixation by spray at these times after launch stresses had been applied.

Although the mechanism and solutions for these fixations worked properly for later anatomical studies of tissues (by Drs. Edwards and Gray), the force and pattern of the spray was such as to create a problem for our analysis of the orientation of roots at the time of each fixation. Motion pictures of a 71-hour fixation spray showed serious displacement effects, particularly for long, slender roots. From the photographic records (see p. 17 on) of all fixed seedlings, we made a study of the resulting positions of the roots and compared the angular measurements with those made earlier on the position of unfixed roots that had grown in our laboratory for the same time periods.

The data in Table II show that the shorter, stiffer roots of the 48-hour seedlings were not injured. The losses to spray of the older seedlings was so serious, however, that the force of the spray must be reduced before further tests are made.

Table II

ORIENTATION OF WHEAT SEEDLING ROOTS FIXED IN JANUARY TEST

(Angles measured in plane of face view of plant)

<u>48-hour</u> - no dislocation by strong fixation spray

Test Pkg.	Angles after Jan. Test			Angles in Earlier Unfixed Plants		
PR	9° - 352°	range	n=11	2° - 356°	range	n=20
LR	M=294.2±8.8	(232-355)	n=12	M=310.7±4.5	(274-336)	n=20
RR	M= 73.2±9.8	(15-136)	n=12	M= 53.9±4.4	(29- 95)	n=20
Erect Pkg.						
PR	M=179.1±4.6	(163-221)	n=12	M=181.0±2.2	(155-202)	n=26
LR	M=227.3±5.5	(197-258)	n=11	M=229.2±1.6	(208-243)	n=26
RR	M=127.6±7.6	(96-180)	n=11	M=133.7±1.6	(119-147)	n=26

<u>64-hour</u> - about 30% dislocation by spray, esp. on lower end & one side

Test Pkg.						
PR	0° - 209°	range	n=9	24° - 332°	range	n=24
LR	M=310.0±16.6	(240-398)	n=9	M=292.0±5.9	(227-331)	n=24
RR	M= 58.3± 8.2	(3- 89)	n=11	M= 69.4±5.0	(37-131)	n=24
Erect Pkg.						
PR	M=183.9±6.2	(151-205)	n=8	M=180.7±2.0	(165-203)	n=22
LR	M=227.4±6.3	(198-251)	n=9	M=228.8±2.4	(208-247)	n=22
RR	M=130.2±5.3	(113-151)	N=9	M=134.3±2.9	(108-161)	n=22

<u>71-hour</u> - estimated 75% dislocation by spray plus contacts from seedlings spaced too closely on 15-unit seed holders.

(data for means of angles include standard errors)

2. TESTS IN DISMANTLED CHAMBERS

The 4 chambers of one of our test packages were removed from the base plate and stripped of their electrical fittings to permit use of individual chambers for separate experiments. Each chamber was attached to a clinostat table so that the growing plants could be either rotated about the axis of the seed holder or placed erect to gravity with the radicle ends of the embryos (see Fig. 1) pointed down.

The holes in the lower end of each chamber were sealed to permit enclosure within it of enough water for saturation of its air with water vapor, as in all growth tests with the system. Temperature was maintained at 77 ± 1° F. by placing the chambers in a dark room with thermostat control of heat and cold air intake as required.

The separate experiments in the dismantled chambers, held either erect or on rotating clinostats at 1 rph, were carried out during studies of ways to improve and standardize the cultural procedures. The results of many such growth tests are recorded in Tables III and IV. The data show both sizes of organs and their orientation in the space around the seed.

V. TECHNIQUES OF LOADING AND PLANTING HOLDERS

The filling of the plastic tubes with a proper mixture of water and a porous medium is essential to germination and growth in any test. Before May, 1965, we had loaded the system by a "wet pack" method that avoided air bubbles and allowed visual control of consistency of the mixture before it was placed in the tubes. Other experimenters were using a "dry pack" method in which the solid material was poured into the tubes and watered there with a syringe. Both methods used progressive capping of the side arms as the system was filled; both methods required an equal and appreciable length of time shortly before the time of planting seeds.

We have recently perfected a dry pack method that permits application of the rubber caps and filling of the tubes with powdered vermiculite in advance of experiment time. A standard mixer-shaker device is used to settle the particles evenly in the side arms as the vermiculite is poured into the upper end (cf. Fig.1).

A few hours before using the system, use a good 10-ml. syringe to inject water slowly through a small hole in each rubber cap, starting with the lowest arm (A 1) of the inverted holder and working slowly up so as to force all air from the open end of the stalk. Immerse the holder in a cylinder of distilled water to allow full hydration of the vermiculite before sealing the open end and planting the soaked seeds.

The first step in preparing the seed is sterilization for 30 seconds in 0.05% $HgCl_2$. The seeds are then rinsed 3 times and at once immersed in aerated distilled water for 4 hours. Tests show that a shorter period of imbibition makes it impossible to select seed with the best embryos and thus to keep the percentage of germination close to 100%.

The proper depth of insertion of soaked seed in the side arm is learned by experience. If the embryo is too near the surface of the rubber, the coleoptile will either grow into the hole beside the endosperm or make such a strong contact with the rim of the side arm as to affect the direction of its later growth. Shallow planting of the seed reduces the area of seed coats through which the water enters the endosperm. The diagram in Figure 1 shows a satisfactory insertion and orientation of the embryo.

VI. PHOTOGRAPHIC RECORDS OF GROWTH FORMS

The direction and form of seedling organ growth can be described fully only by orientation measurements in 3 dimensions. A permanent record must be made at the close of each experiment before the seedlings are removed from the holder for measurements of organ length and later analyses of the tissues. This record is being taken in color on 35 mm. film as soon as the growth chamber has been opened and notes have been made of possible deformities due to contacts of roots with other objects. Each picture is a record of the 4 or 5 seedlings in one of the 3 vertical rows on a seed holder (cf. Fig. 1). The 3 exposures of each set of plants are used as lantern slides with consequent high accuracy in measurements from sharp, enlarged projections.

A combined camera stand and stage for the seed holder has been designed, fabricated and found adequate for taking good pictures rapidly. Fittings on the stage end of the rack hold a set of seedlings erect against a black background. A Pentax camera is attached at the other end of the rack with a fixed focus and 16 stop to provide a deep field of view in each picture.

A mirror on the left of the seed holder reflects a side view of each seedling when the row of plants is adjusted for face views of them (see Figure 3). An electronic flash beside the camera and coupled to the shutter release provides identical, correct exposures and a brief, intense lighting that does not injure the seedling roots.

The growth form records of 2 seedlings are shown by traced sketches in Figure 3 as the organs appear in wall projection of 2 color slides. The tracings are from projected pictures of representative seedlings after 72 hours growth, one on a clinostat and the other erect to gravity on a separate seed holder.

A second rack and camera system is needed for a second picture with mirror on the right side of seedlings in important experiments. The tip of the righthand secondary root is sometimes hidden (cf. record sheet on p. 24) behind the side arm if only a mirror view of the left side of the plant and arm is available. The second picture on a second film will also serve as insurance against loss of any one film during processing.

The exact form of each root is preserved after removal of the seedling holder from the growth chamber if the pictures are taken in a room with relative humidity at 85%. No higher content of water vapor is required to prevent wilting of the root tips.

COORDINATE METHOD FOR ORIENTATION ANGLES

Data
(in mm.)

	x	y	z
C	- 6	27	22
PR	-26	-38	-65
LR	-36	35	-29
RR	36	10	-15

	x	y	z
C	0	16	4
PR	2	-62	-12
LR	-40	-23	-11
RR	45	-22	0

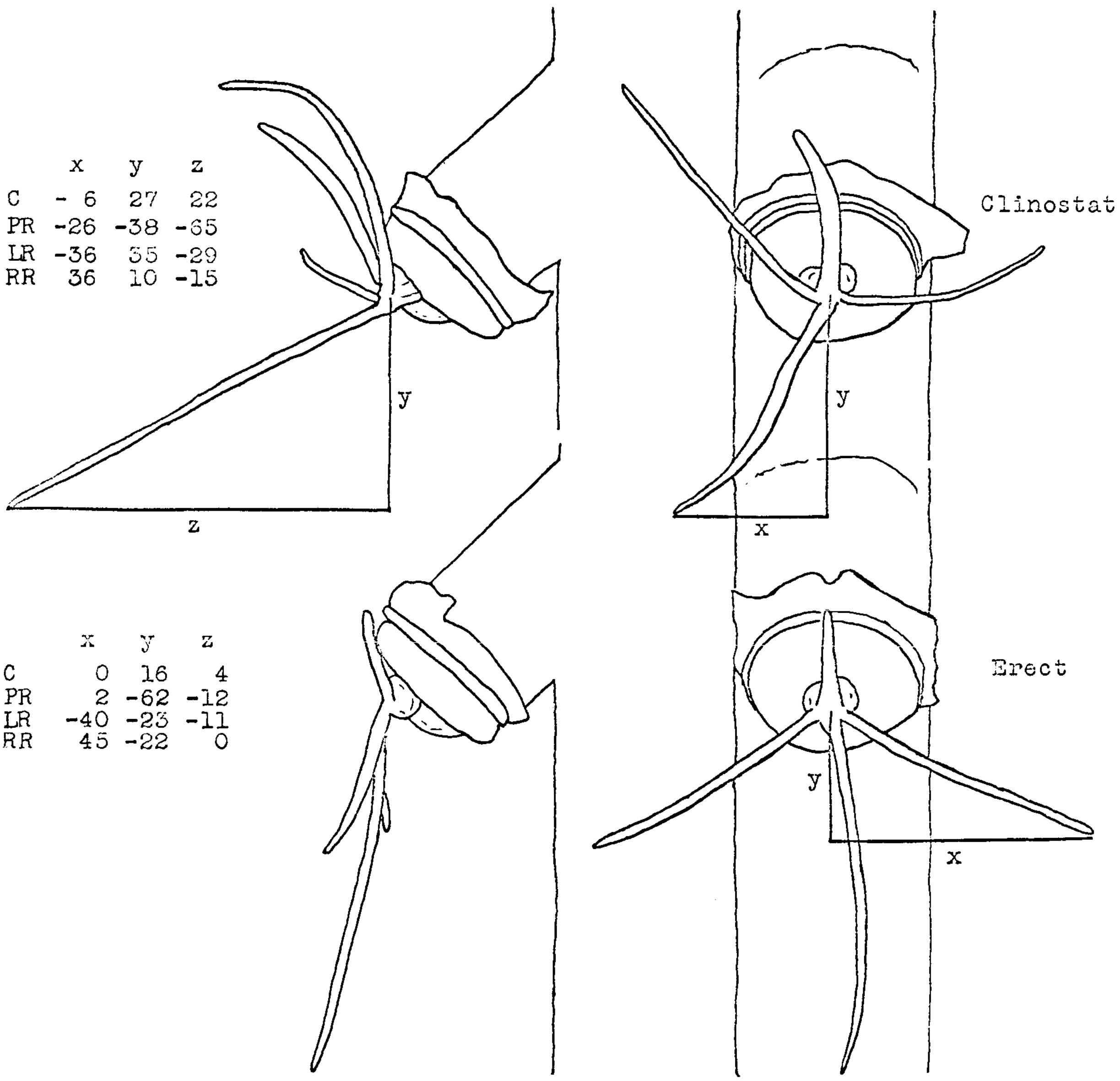

Figure 3. Tracings of Projected Pictures of Seedlings (with diagrams and data to show method of collecting orientation measurements)

VII. COLLECTION OF ORIENTATION DATA

The orientation of each seedling organ is described in quantitative terms by means of 2 plane angles that can be computed from measurements taken on projections of seedling pictures, as illustrated in Figure 3. The combination of the 2 angles gives the position of the organ tip in 3 dimensions in relation to the axis of the plant. If experience shows a need to make an analysis that will take into consideration the curvatures in many organs, the photographic records will be available for this purpose.

One angle is that formed in a projected face view of a seedling between the Y axis through the base of the organ and a line through the base and tip of the coleoptile or a root. The coordinates for computing this angle are x and y (see Fig. 3), with the quadrant determined by the arithmetic signs of the data.

The second angle is formed in the same way in the projected side view of the seedling, with coordinates y and z as the data for computations. This angle measures the displacement of the organ tip from the vertical axis through the organ's base, either toward the camera (left in Fig. 3) or away from the camera.

Measurements of the 3 coordinates for each organ tip are made from the color slide projection on a large sheet of graph paper that is fastened to a wall about 8 feet from the projector. With the vertical lines of the paper oriented with the seed holder axis in the picture projection, the x, y and z coordinates are taken with dividers and scaled on an illuminated part of the paper.

The x and y data are recorded as + or - from the origin as in ordinary usage. For roots, the z coordinate is considered negative if it is measured left of the 0,0 point on the vertical (Y) axis in the side view of the seedling; for coleoptile curvatures toward or away from the camera, the z distance is recorded as positive if it extends left of the Y axis, since the wheat coleoptile seldom bends toward the side arm.

The sample work sheet on page 22 shows the nature and variety of the coordinates obtained. This tally system is used beside the wall projection as the investigator measures and records the data. The measurements of organ lengths in mm. had been entered earlier on the work sheet immediately after the photograph had been taken. The enlargement by projection can be estimated roughly from the ratio of a straight coleoptile measurement in mm to the y coordinate in tenths of an inch for the same organ that is known to have grown straight when the x and z coordinates are both zero.

SAMPLE WORK SHEET

Size and Coordinates of Seedling Organs

Experiment *Use of All-Plastic Holders* Seeds *'64 Best* Medium *Vermiculite*

Stalk	Row	No.	CO	x	y	z	PR	x	y	z	LR	x	y	z	RR	x	y	z
A	A	1	7	-1	18.6	.8	39	-10	-51.8	-33	15	-23.1	-16	-3	17	29.4	-10.7	-4
		2	10	-1.7	18.2	1	47	-12	-67	0	28	-42	-23.8	12.3	25	38.6	-25	-3.5
Hours		3	9	.2	14.9	1.4	37	-16.4	-62.6	-13.3	22	-29	-14.2	6.2	21	30.2	-13.2	7.3
72		4	6	-1	10.4	1.2	34	-1	-51.6	-24.3	12	-19.8	-6.7	5.1	12	22.5	-8	-1.1
		5																
Position	B	1	4	.4	8	1.5	26	-6	-39	-22	19	-31.3	-12.6	2.5	17	27.5	-12.8	0
Erect		2	12	0	22.6	1	37	-10	-60	-22	31	-39.4	-34.3	-18.1	25	40.5	-19	-1
		3	6	0	10	.4	25	8	-44	-5	17	-20.6	-16.8	-14.4	14	19.8	-12.3	7
Date		4	5	2	5.1	2.2	27	3.5	-46	-18	16	-23.2	-15.2	4.1	16	23.8	-14	6
6/26/65		5																
	C	1	6	0	11.1	0	35	-9	-55	-25	20	-30.7	-19.6	-.5	19	30.6	-13.8	-4.9
Load		2	6	.8	10.7	2.8	42	-24.5	-65	-19.8	18	-29.3	-14.6	7	20	27	-14	-8
Shaker		3	10	-.5	18.1	1.6	37	-16	-56	-15	21	-29	-14.9	16.7	21	28.4	-14	21
Pack		4	23	1	39.3	5	54		T.W.		27	-25.2	-18	25	23	40.3	-14.7	0
Very Fine		5																
Ver.	Mean		8.7				36.7				20.5				19.2			

Stalk	Row	No.	CO	x	y	z	PR	x	y	z	LR	x	y	z	RR	x	y	z
	A	1	4	-1.1	7	1.1	36	-25.5	-36	-23.2	21	-28.2	6	-14.8	24	34.7	12	-4
		2	7	-2.7	10.2	4	28	-19	11	-37	14	-24	.5	-8.4	16	24.5	-6.7	-5
Hours		3	3	.1	5.6	.4	27	26.7	-26.2	-21.7	14	-25.3	2	-5.8	17	27.6	-10	-3.4
72		4	3	.3	5.3	1.1	28	-15.2	-37.8	-25.3	—	0 0 0			17	21.9	-18.4	-7.7
		5	4	-1.3	6.7	.6	24	2	-30.7	-29.1	21	-36	-6.2	-13	17	26.5	-1	-8.2
Position	B	1	7	-4.8	10.8	3.2	31	33	-30	-42	15	-27	-.2	-8.2	19	25.3	4	-10.1
Clinostat		2	17	-8.4	24.8	11.3	27	-12	-10.3	-41.7	30	-44.6	19.6	12	23	33.7	16.3	-13.8
		3	4	-1.1	7.3	1.2	27	-8.2	-34.1	-27.7	18	-28.2	-10	1.6	15	16.4	15.8	-4
Date		4	6	-.5	8	4.1	29	-2	-25.4	-40.5	16	-27.8	-10	-4	17	29	-6.3	-4.4
5/27/65		5	2	-.5	4.4	.6	26	-38	3.9	-21.3	17	-22.8	14	1.5	21	33	13	?
	C	1	5	-2.6	7.9	.9	31	-13.9	-46.6	-17.4	12	-20	-6.6	-6	16	20.7	16.5	-7.3
Load		2	12	-4.3	16.2	11	36	-16.8	-23.3	-39	27	-28	33.3	5.5	19	31	3.4	?
Mild		3	5	0	9.5	2	30	-20.5	-43	-21	14	-22.3	7.9	-5	23	18.1	25	-16.9
Shake		4	6	-2.7	9.5	3.9	24	-13	-15	-33	20	-34	-2	-11.2	17	5.9	27.4	-5
(Yellow		5	5	.8	7.2	.9	27	10.3	-39	-16	17	-25	9.3	10	22	33	-4.3	-12
Fine Ver.)	Mean		6.0				28.3				18.3				18.9			

Stalk	Row	No.	CO	x	y	z	PR	x	y	z	LR	x	y	z	RR	x	y	z
	A	1	5	3	8.8	2.4	33	15	-53	-19.2	11	-19	5.4	-5.5	19	28.5	4.3	-5.2
		2	9	2.5	15.6	4.1	33	-4	-40.3	-40.1	30	-18.4	32	25.5	24	28.7	26.3	2
Hours		3	5	1.3	9.7	1	25	6	-26.1	-28.4	15	-28.7	4	-2.2	16	28.4	3.1	?
60		4	9	1.1	13.7	0	32	18.3	-33	-39.5	21	-36.3	3.5	0	22	32.6	9.3	0
		5	7	-1.9	11.4	2.1	30	3	-21	-44	16	-27.1	10	-4	17	27	7.4	-12
Position	B	1	6	-3	11	2.1	24	12.3	-12.4	-33	26	-41	-13.5	-22	14	24.6	-4.8	7.9
Clinostat		2	5	-7.6	6.3	2.8	31	28.6	-50	-1.7	—	0 0 0			—		0 0 0	
		3	7	3	10.3	.8	33	31	-29	-36	18	-23	23.4	-4.6	14	25.9	1.5	-5
Date		4	6	1.5	9	1.3	30	-20	-27.1	-41	18	-21.6	20	-3	19	32.3	.5	4.8
7/1/65		5	5	-1.6	7.9	0	23	13.2	-34	-20	15	-21	12	1	17	21.4	16	3.5
	C	1	4	-1.5	6.3	1.1	28	21.3	-32	-26.7	21	-36	5	5	9	13.5	7	-2
Load		2	4	-1.3	7	3.1	22	20.3	-26.8	-18	10	-12	8	-9.2	18	30.8	11.2	-7
Shaker		3	5	-.1	8	1	29	-13.3	-38	-32.9	13	-24	5	-6	13	23	5	1
Pack		4	11	4.6	18.5	-1	39	18	-56.4	-37	26	-27	33	-11	28	26.4	26.5	17.8
Very Fine		5	4	0	7.1	1	31	26.5	-45.2	-22.6	16	-28	-5	-10	15	29	3	-3.4
Verm.	Mean		6.1				29.5				18.3				16.8			

T.W.=touched chamber wall   0 0 0 is code for computer=no record

VIII. DATA PROCESSING BY COMPUTER

We are using computer programs to obtain for each seedling organ 2 angles which describe the position of the organ as recorded by photography. The angles are computed separately from the x and y coordinates for the face view angles and from the z and y data for the side views. An angle is derived from the x/y or z/y ratio as the tangent of the desired angle. The angle obtained from the side view reflects to some extent the face view angle but it is valid for comparative evaluation of the curvature in the third dimension.

The computer program for coleoptile curvatures supplies a face view angle that reads clockwise in degrees from the erect (0^o) position. It also computes a side view angle that is measured as positive (toward the camera) or negative from the +Y axis. The sample record sheet (next page) shows typical results from the data on the work sheet (p. 22) for the same 3 sets of seedlings. The complete program for these computations is listed in the appendix below. The face view and side view angles for the 2 seedlings in Figure 3 (p. 19) are found to be respectively 347.5 and 39.2 for the clinostat experiment and 0 and 14.0 for the erect plant.

The program for root orientation computes the same 2 angles for each of the 3 roots. Face view angles read clockwise from 0^o to 360^o. The side angles read from 0^o to 180^o up from the -Y axis, since the reference position of roots under gravity stimulation is "down ($=180^o$). Side angles are recorded as positive if the root curvature is toward the camera and negative if toward the holder. See next page for data computed from work sheet data.

SAMPLE RECORD SHEET

Size and Orientation Angles of Seedling Organs

Experiment Use of All-Plastic Holders Seeds '64 best Medium Vermiculite

Stalk	Row	No.	CO	Face	Side	PR	Face	Side	LR	Face	Side	RR	Face	Side
A	A	1	7	354.6	4.3	39	190.9	32.5	15	235.3	10.6	17	110.0	20.5
		2	10	354.7	3.1	47	190.2	0	28	240.5	-27.4	25	122.9	8.0
Hours		3	9	0.8	5.4	37	194.7	12.0	22	243.9	-23.7	21	113.6	-29.0
72		4	6	354.5	6.6	34	181.1	25.2	12	251.3	-37.4	12	109.6	7.8
		5												
Position	B	1	4	2.9	10.6	26	188.7	29.4	19	248.1	-11.3	17	115.0	0
Erect		2	12	0	2.5	37	189.5	20.1	31	229.0	27.8	25	115.1	3.0
		3	6	0	2.3	25	169.7	6.5	17	230.8	40.6	14	121.8	-29.7
Date		4	5	21.4	23.3	27	175.6	21.4	16	236.8	-15.2	16	120.5	-23.3
6/26/65		5												
	C	1	6	0	0	35	189.3	24.4	20	237.4	1.5	19	114.3	19.5
Load		2	6	4.3	14.7	42	200.7	16.9	18	243.5	-25.7	20	117.4	29.7
Shaker		3	10	358.4	5.1	37	195.9	15.0	21	242.8	-48.4	21	116.2	-56.4
Pack		4	23	1.5	7.3	54	T.W.		27	234.5	-54.3	23	110.0	0
Very Fine		5												
Vermiculite	Mean		8.7 ± 1.5	4.1 ± 1.7	+7.1 ± 1.9	36.7 ± 2.5	187.8 ± 2.7	+18.5 ± 2.9	20.5 ± 1.0	239.5 ± 1.9	27.0 ± 4.6	19.2 ± 1.2	115.5 ± 1.3	18.9 ± 4.7

Stalk			C	f	s	P	f	s	L	f	s	R	f	s
	A	1	4	351.1	8.9	36	215.3	32.8	21	282.0	112.1	24	70.9	161.6
		2	7	345.2	21.4	28	300.1	106.6	14	271.2	93.4	16	74.7	143.3
Hours		3	3	1.0	4.1	27	134.5	39.6	14	274.5	109.0	17	109.9	18.8
72		4	3	3.2	11.7	28	201.9	33.8	—			17	130.0	22.7
		5	4	349.0	5.1	24	176.3	43.5	21	260.2	64.5	17	92.2	83.0
Position	B	1	7	336.0	16.5	31	132.3	54.5	15	269.6	88.6	19	81.0	111.6
Clinostat		2	17	341.3	24.5	27	229.4	76.1	30	293.7	-148.6	23	64.2	139.7
		3	4	351.4	9.3	27	193.5	39.1	18	250.5	-9.2	15	46.1	165.8
Date		4	6	356.4	27.1	29	184.5	57.9	16	250.2	21.8	17	102.3	34.9
5/27/65		5	2	353.5	7.8	26	275.9	100.4	17	301.6	-174.0	21	68.5	– hidden
	C	1	5	341.8	6.5	31	196.6	26.5	12	251.7	42.3	16	51.4	156.1
Load		2	12	345.1	34.2	30	215.8	59.1	27	319.9	-170.7	19	83.7	– hidden
Mild		3	5	0	11.9	30	205.5	26.0	14	289.5	147.7	23	35.9	145.9
Shake (Yellow		4	6	344.1	22.3	24	220.9	65.6	20	266.6	79.9	17	12.2	169.7
		5	5	6.3	7.1	27	165.2	22.3	17	290.4	-133.0	22	97.4	70.3
Fine Ver.)	Mean		6.0 ± 1.0	10.4 ± 1.8	+14.6 ± 2.4	28.3 ± 0.8		+51.9 ± 6.8	18.3 ± 1.4	276.5 ± 5.6	99.6 ± 14.1	18.9 ± 0.8	74.7 ± 7.8	109.5 ± 15.7

Stalk			C	f	s	P	f	s	L	f	s	R	f	s
	A	1	5	18.8	15.3	33	164.2	19.9	11	285.9	134.5	19	81.4	129.6
		2	9	9.1	14.7	33	185.7	44.9	30	330.1	-141.5	24	47.5	-175.8
Hours		3	5	7.6	5.9	25	167.1	47.4	15	277.9	151.2	16	83.8	hidden
60		4	9	4.6	0	32	151.0	50.1	21	275.5	180.0	22	74.1	180.0
		5	7	350.5	10.4	30	171.9	64.5	16	290.3	138.2	17	74.7	121.7
Position	B	1	6	344.7	10.8	24	135.2	69.4	26	251.8	585	14	101.0	-58.8
Clinostat		2	5	309.7	24.0	31	150.2	1.9	–			–		
		3	7	16.2	4.4	33	133.1	51.1	18	315.5	168.9	14	86.7	106.7
Date		4	6	9.5	8.2	30	216.4	56.5	18	312.8	171.5	19	89.1	-96.0
7/1/65		5	5	348.6	0	23	158.8	30.5	15	299.7	-175.3	17	53.2	-167.8
	C	1	4	346.6	9.9	28	146.4	39.8	21	277.9	-135.1	9	62.6	164.1
Load		2	4	349.5	23.9	22	142.9	33.9	10	303.7	131.0	8	70.0	148.0
Shaker		3	5	359.3	7.1	29	199.3	40.9	13	281.8	129.8	13	77.7	-168.8
Pack Very Fine		4	11	14.0	-3.2	39	162.3	33.3	26	320.7	161.6	28	44.9	-146.2
		5	4	0	8.0	31	149.6	26.6	16	259.9	63.4	15	84.1	131.4
Vermic.	Mean		6.1 ± 0.5	12.7 ± 3.0	9.7 ± 1.9	29.5 ± 1.2		+40.7 ± 4.5	18.3 ± 1.6	291.7 ± 6.2	140.0 ± 10.0	18.8 ± 1.5	73.6 ± 4.4	138.1 ± 9.9

The Dartmouth GE high speed computer is available to an investigator on a time-sharing plan by which he can use the system daily at his convenience in the afternoon or evening. We send in our data from a teletype on the ground floor of our Gilman Life Sciences Laboratory. The programs for our orientation angles and for statistical analyses of our results are stored in the computer and have only to be called up for use just before data are typed in. Results are returned to the same machine within a minute or two after a set of data has been concluded.

The program for computation of our root angles is listed in the appendix of this report. To illustrate the convenient form in which results are delivered, the data return for the root angles of the 2 seedlings in Fig. 3 is reproduced here. BIOSAT is the call name of the root program in the computer's file. The data for the clinostat plant were sent in first, in the form shown at the end of the program. The face view angles are listed in the first column.

```
BIOSAT     14:06     JULY 22,1965

PR     214.4     59.7
LR     314.2     140.4
RR     74.5      123.7

PR     178.2     11
LR     240.1     25.6
RR     116.1     0

OUT OF DATA IN 110

TIME:    1 SECS
```

IX. EXPERIMENTAL RESULTS

1. TESTS WITH PREPROTOTYPE HARDWARE

A summary of experimental data and computations from our photographic records is listed in Tables III and IV on the following pages. The entries represent work done during 1965 with the growth chambers and other hardware essentially as they will be operating for the orbital experiment.

The results in the growth tests of wheat were not equally satisfactory during the first 3 months of 1965. The growth was often incomplete because water was lacking to the seedlings during the later hours of the growth period. The better techniques used for the experiments reported in Table IV improved the percentage of germination and sustained growth but the best methods and materials were not used until the month of June.

In spite of the relatively short coleoptiles and roots in many of these tests with wheat, the patterns of organ orientation were much alike for all the experiments. The mean angles for the erect plants and the nature of the variations in positions taken by individual organs remained within narrow limits. The patterns of displacement and curvature in the organs that developed on horizontal clinostats were also more nearly alike than were the sizes of the organs. This base line data promises to be appropriate for evaluation of a flight experiment. The measurable differences in orientation features between plants erect to gravity and those grown without geotropic responses show that the orientation aspects of growth at zero gravity can probably be appraised in quantitative terms without dependence on maximum lengths of roots and coleoptiles.

Table III. SIZE AND ORIENTATION ANGLES OF SEEDLING ORGANS - Part 1

(means of length in mm; mean angles from coordinates of organ tips)

Date 1965	Hrs.	Med.	Nos.	Coleoptile Lgth	Coleoptile Face	Coleoptile Side	Primary Root Lgth	Primary Root Face	Primary Root Side	Left Root Lgth	Left Root Face	Left Root Side	Right Root Lgth	Right Root Face	Right Root Side
ERECT															
3-12	48	C & V	12	2.4	2.9	+2.0	20.8	181.7	8.7	10.6	240.3	14.4	11.0	119.1	14.1
"	"	"	12	2.7	1.7	+4.0	19.9	182.2	13.2	15.6	240.3	27.5	16.8	119.1	10.0
"	"	"	12	2.3	3.4	+3.9	18.3	176.6	8.0	10.8	236.7	10.8	10.5	132.9	16.7
3-18	64	"	11	4.1	3.1	+2.4	21.1	189.0	18.3	19.3	239.8	29.5	17.6	126.6	27.2
"	"	"	12	3.7	1.0	+2.9	20.2	180.3	18.7	16.3	235.9	31.1	14.8	124.2	24.8
3-23	72	"	12	4.4	6.6	+6.0	29.3	181.2	9.3	15.7	226.0	22.0	16.1	126.6	23.5
"	"	"	11	3.9	0.5	+1.8	22.5	174.9	14.5	15.3	228.6	11.7	13.0	124.2	17.1
	mean angles				2.7	+3.3		180.8	13.0		235.3	21.0		124.7	19.1
CLINOSTAT															
2-8	48	BrVer	9	3.3	5.3	6.5	18.8		76.8	11.2	292.3	129.0	12.2	59.3	136.0
"	"	"	12	2.6	4.4	+5.7	16.0		57.1	8.6	292.8	141.7	7.6	73.2	104.1
"	"	"	13	3.0	3.8	+4.4	17.4		+91.5	11.7	276.6	113.0	11.0	63.4	154.2
2-17	"	C & V	12	3.0	3.7	1.7	19.7		103.4	11.4	276.1	+ 91.6	11.5	73.2	128.8
"	"	"	11	3.8	4.4	+1.8	21.4		+88.6	12.3	302.0	154.6	14.0	59.2	163.3
mean	48		11.4	3.1	4.3	4.0	18.7		83.5	11.0	288.0	126.0	11.3	65.7	137.3
3-4	64	C & V	14	3.0	4.4	- .9	25.4		+67.6	13.3	292.9	+140.5	13.7	77.0	130.8
3-9	"	Coll.	15	4.3	17.8	+5.5	25.1		55.0	16.0	309.4	151.7	16.1	69.7	145.3
3-18	"	C & V	15	6.4	12.8	+12.3	22.4		49.5	18.5	289.7	108.6	19.7	67.1	+140.0
mean	64		14.7	4.6	11.7	6.2	24.3		57.4	15.9	297.3	133.6	16.5	71.3	138.7
2-9	72	BrVer	10	8.4	19.4	10.2	30.7		77.9	22.2	291.1	+104.7	22.5	77.2	114.2
"	"	"	7	8.0	13.4	+ 8.0	34.0		82.9	22.4	312.5	145.2	23.1	64.4	147.2
2-18	"	C & V	9	12.9	8.9	+14.2	26.8		50.6	23.9	295.0	108.4	23.6	66.1	128.9
"	"	"	12	10.5	12.5	+12.9	31.0		112.9	24.3	287.2	119.1	24.0	78.4	131.9
3-12	"	"	15	7.3	18.5	+15.5	26.6		+ 53.6	16.2	295.3	+133.6	17.1	68.1	115.7
3-23	"	"	15	6.8	10.8	+19.6	28.5		+ 35.7	21.3	283.0	+106.7	22.1	70.9	117.7
3-29	"	"	12	4.9	6.9	+14.8	34.8		+ 27.1	16.8	283.7	+105.7	17.8	85.5	82.2
"	"	"	11	3.9	6.9	+19.1	34.5		48.7	15.6	277.4	114.6	16.4	81.1	96.3
"	"	YwVer	15	5.4	10.8	+13.1	37.7		29.9	22.9	293.8	128.8	23.2	69.7	84.7
"	"	BrVer	15	5.1	9.3	+15.6	30.4		+ 36.5	18.2	266.7	+ 89.2	18.8	75.8	+137.5
mean	72		12.1	7.3	11.7	14.3	31.5		55.6	20.4	288.6	115.6	20.9	73.7	115.6
Test at ARC															
ERECT															
#5	48F	Cell.	10	2.5	1.0	+ 5.9	18.6	181.2	21.6	7.8	229.4	20.7	6.2	127.0	+ 2.8
8	64F	BrVer	13	9.2	2.4	+13.3	44.3	186.2	-13.1	23.7	230.5	25.7	23.5	132.7	23.3
9	71F	"	14	14.1	.9	+10.0	36.5	184.6	3.6	30.8	235.5	- 9.9	33.4	153.5	- 4.0
2	72	"	12	4.3	5.9	+ .8	18.1	175.7	18.6	15.4	228.7	21.8	14.0	133.6	14.0
4	"	Cell.	10	3.9	5.7	+ 4.1	16.6	189.1	13.3	13.7	235.5	28.2	13.1	133.2	17.5
11	"	BrVer	10	6.1	1.7	+ 2.5	16.4	202.3	13.7	11.7	240.9	33.2	13.2	125.3	15.6
	mean angles				2.9	+ 6.1		186.5	14.0		233.4	23.3		134.2	12.9
CLINOSTAT															
#5	48F	Cell.	12	4.0	11.2	11.8	18.5		+ 61.1	12.1	294.1	137.1	11.9	71.9	+141.3
12	64F	BrVer	15	6.9	12.5	+21.7	27.2		48.8	21.0	305.3	113.8	21.5	53.9	143.2
1	71F	"	12	10.0	20.4	14.3	32.5		75.2	20.8	307.6	137.0	21.5	42.6	136.6
3	72	"	10	6.6	9.9	11.1	31.4		53.8	19.9	259.2	+ 77.0	20.9	78.1	+114.0
7	"	Cell.	10	10.0	15.3	3.7	35.2		67.6	22.3	307.6	147.4	20.9	54.6	143.6
10	"	BrVer	6	16.5	8.4	18.0	43.3		+ 52.4	26.2	265.5	96.3	25.4	50.1	+149.5
	mean angles				13.0	13.4			59.8		289.9	118.1		58.5	138.0

Notes: Mean angle curvature of coleoptile is not mean angle position
- for a side angle indicates all bent toward stalk
+ " " " " " " away from stalk (toward chamber wall)

Table III. SIZE AND ORIENTATION ANGLES OF SEEDLING ORGANS - Part 2

(means of length in mm; mean angles from coordinates of organ tips)

Date 1965	Hrs.	Med.	Nos.	Coleoptile Lgth	Coleoptile Face	Coleoptile Side	Primary Root Lgth	Primary Root Face	Primary Root Side	Left Root Lgth	Left Root Face	Left Root Side	Right Root Lgth	Right Root Face	Right Root Side
WHEAT-ERECT															
5-7	48	FiVer	12	2.8	5.3	+10.2	23.8	175.3	7.7	11.0	239.7	16.2	10.8	118.9	21.5
7-1	60	VFVer	12	5.9	3.1	+ 4.8	25.5	185.7	16.4	16.1	235.5	16.7	14.9	115.4	22.7
"	"	"	12	6.7	4.0	+ 7.6	27.6	184.2	15.4	16.8	240.5	17.1	17.3	122.1	17.5
4-15	72	FiVer	11	4.7	3.8	+ 3.5	26.7	178.9	+ 7.9	16.1	229.3	+20.6	14.4	128.7	14.1
5- 3	"	"	11	6.0	3.4	+11.1	26.9	183.8	9.8	18.2	232.3	24.8	19.1	127.7	13.5
6-26	"	VFVer	12	5.9	2.0	+ 2.6	28.2	187.0	12.0	15.3	239.0	20.1	17.0	117.8	17.4
"	"	"	12	8.7	4.1	+ 7.1	36.7	187.8	+18.5	20.5	239.5	27.0	19.2	115.5	18.9
"	"	"	12	9.0	4.7	+11.0	35.0	182.0	11.2	20.1	236.8	30.5	20.5	116.8	19.4
6-20	92	FiVer	11	6.8	2.9	+ 9.6	30.6	178.0	5.9	21.4	232.8	21.7	20.5	126.9	17.2
"	"	C & V	12	7.4	7.0	+ 7.2	28.3	181.5	12.6	19.8	234.2	16.8	20.1	128.3	18.0
		mean angles			4.0	+ 7.5		182.4	11.7		236.0	21.2		121.8	18.0
WHEAT-CLINOSTAT															
5-2	48	FiVer	15	2.9	8.7	+ 8.7	20.7		+42.2	7.7	270.8	85.0	8.3	81.3	91.3
"	"	"	15	3.1	3.2	+12.1	20.7		48.3	8.5	262.6	61.5	8.1	96.7	64.9
mean	48		15	3.0	6.0	+10.4	20.7		45.3	8.1	266.7	73.3	8.2	89.0	78.1
7-1	60	VFVer	15	6.5	15.3	6.2	28.9		+43.0	16.9	297.3	138.2	17.9	77.7	136.5
"	"	"	15	6.1	12.7	9.7	29.5		+40.7	18.3	291.7	140.0	16.8	73.6	138.1
mean	60		15	6.3	14.0	8.0	29.2		+41.9	17.6	294.5	139.1	17.4	75.7	137.3
4-15	72	FiVer	13	10.0	13.4	+21.5	36.8		+39.7	22.3	293.1	+126.1	22.7	83.4	123.9
4-26	"	"	14	5.2	7.9	+ 7.0	29.9		34.9	15.4	281.1	120.1	17.2	61.8	159.5
"	"	"	15	8.6	10.3	+15.8	37.7		+30.3	19.4	287.0	115.9	20.7	69.0	141.4
5- 3	"	"	15	4.4	7.2	+15.3	29.2		+31.1	17.2	284.6	114.7	17.6	69.7	123.5
"	"	"	15	4.4	8.6	+16.8	29.7		44.2	17.1	273.2	90.7	17.2	85.2	106.5
5-10	"	C & V	15	5.2	11.7	+23.2	30.0		27.7	18.7	279.1	105.7	19.4	75.1	130.1
5-27	"	"	15	5.4	6.4	+19.8	30.9		+37.2	17.8	279.6	106.3	18.6	74.5	106.4
"	"	FiVer	15	6.0	10.4	+14.6	28.3		+51.9	18.3	276.5	99.6	18.9	74.7	109.5
6- 4	"	"	13	9.7	19.8	15.2	33.8		61.1	22.1	280.5	106.5	24.0	79.5	112.9
"	"	C & V	12	6.3	11.3	5.3	27.2		+60.3	16.8	295.7	+130.7	16.4	77.6	121.6
6-11	"	"	15	4.8	11.6	+ 7.2	26.9		+54.0	14.1	277.7	+ 92.1	16.2	67.0	136.3
"	"	FiVer	15	5.5	13.8	+14.1	29.8		29.0	19.4	279.2	122.1	19.8	64.6	132.6
6-26	"	VFVer	15	5.5	10.6	5.5	25.2		68.3	16.1	283.6	+112.1	15.7	81.7	104.5
"	"	"	14	10.6	15.5	10.4	37.4		55.4	15.1	293.5	132.4	18.9	89.2	93.3
mean	72		14.4	6.5	11.3	13.7	30.9		44.7	17.8	283.2	112.5	18.8	75.2	121.6
6-20	92	FiVer	14	19.3	17.5	12.9	37.6		45.8	21.6	282.9	+103.0	22.4	74.4	131.2
BARLEY-ERECT															
5-21	48	C & V	12	3.1	2.9	+14.5	16.8	181.2	7.4	10.1	192.5	18.0	10.1	153.7	18.4
"	"	"	12	3.5	6.2	+ 9.3	17.9	179.1	5.7	13.1	191.6	21.3	12.8	159.1	15.7
BARLEY-CLINOSTAT															
5-21	48	C & V	15	7.3	10.0	+26.1	23.9		66.7	18.9	257.7	77.9	21.9	79.5	95.8
RYE-ERECT															
5-14	48	C & V	9	3.4	1.7	4.2	27.1	183.7	11.4	12.0	199.9	13.7	12.6	169.7	6.3
"	"	"	11	3.9	5.3	3.2	33.1	182.2	4.6	18.4	188.5	11.3	16.3	160.2	20.2
RYE-CLINOSTAT															
5-14	48	C & V	14	6.7	15.4	10.0	34.2		52.0	25.0	254.3	78.5	23.9	129.5	79.9

Notes: Mean angle curvature of coleoptile is not mean angle position
+ for a side angle indicates all curvatures were away from seed holder
FiVer means Fine Vermiculite; VFVer means Very Fine Vermiculite
C & V means a mixture of powdered cellulose and fine vermiculite

The greatest need for improvement in the data for erect and clinostat growth lies in developing as much uniformity in growth rates as the biological variation in the wheat embryos will permit. More attention to precision in loading the seed holders with an optimum mixture of vermiculite and water should improve the base line data as the experimentation is continued.

The results of a few comparable tests with barley and rye seedlings grown in the same hardware for 48 hours are included in Table IV. The absence in these plants of a well defined primary root and a pair of secondary roots growing at characteristic angles with the longest root prevented strict comparisons with wheat. The tests were closed at 48 hours to permit use of the first roots to appear as barley and rye germinated rapidly at 77° F. Only the 3 longest roots were used in the analysis of orientation angles.

Although these longer roots showed a lack of precise pattern in direction of growth when the plants were erect to gravity, the changes in orientation angles when grown on a horizontal clinostat were very similar to the corresponding changes in wheat seedlings. The side view angles of the longest root were 8 to 10 times as large on the clinostat while the corresponding factor for wheat is only about 4X. The 2 "lateral" roots of barley and rye, however, responded to removal of the tropistic effects of gravity by about the same factor (6X) as in wheat. The corresponding changes in the face view angles were about the same among the 3 types of seedlings. Table IV also shows comparable changes in the face and side view angles of the coleoptiles of wheat, rye and barley when grown on clinostats rather than erect.

The results of this limited experimentation with other grass-type seedlings make it clear that wheat is a better test plant but that the differences in growth form of its seedling organs between experiments with and without the tropistic effects of gravity are not peculiar to wheat. The supplies and distribution of growth regulators within the grass seedling appear to be much the same between genera although 2 experiments with Avena seedlings on clinostats suggest that the regulation of growth in its seedling organs may be quantitatively different in relation to geotropic reactions.

2. WHEAT SEEDLINGS IN SOIL

As a check on the validity of orientation of roots and coleoptiles grown in our seed holders in comparison with the growth form of young plants rooted in soil, a series of 30 seedlings were grown for 72 hours from seeds planted in moist soil to a depth of about 1 cm. The seeds were pre-soaked as in holder tests and set erect in the soil with the embryo end down. Most of the soil was washed from the roots at the close of the test. The seedlings were at once floated above a white surface in shallow water to permit retention of the growth form of each as the water was slowly pipetted away.

Only the face angles of orientation of roots and coleoptiles could be measured (with a goniometer) but they are comparable with the corresponding angles computed for the erect wheat seedlings represented in Tables III and IV. The data for these angles and the lengths of organs are shown in Table V beside measurements of growth in 2 other systems.

Table V

Orientation of Wheat Seedlings Grown by Different Systems

(shown by mean angles read clockwise from 0° for erect position*)

	Coleoptile Mean Lgth mm	Coleoptile Face Angle	Primary Root Mean Lgth mm	Primary Root Face Angle	Left Root Mean Lgth mm	Left Root Face Angle	Right Root Mean Lgth mm	Right Root Face Angle
ERECT								
In Soil	25.8 ± 1.9	4.5 ± 0.9	65.4 ± 4.2	184.1 ± 2.3	41.9 ± 2.2	229.4 ± 2.0	43.1 ± 2.5	127.9 ± 2.8
In Holders	6.1 ± 0.8	3.6 ± 0.7	29.3 ± 1.9	182.2 ± 1.7	17.3 ± 0.9	233.1 ± 2.0	17.0 ± 1.0	122.5 ± 2.1
On Agar	9.5 ± 0.6	5.7 ± 0.9	31.5 ± 1.7	183.5 ± 1.7	23.4 ± 1.2	221.8 ± 2.3	23.4 ± 1.2	139.7 ± 1.7
CLINOSTAT								
In Holders	6.9 ± 0.5	10.5 ± 0.8	31.2 ± 0.8	--	18.9 ± 0.6	285.4 ± 2.0	19.7 ± 0.6	74.6 ± 1.5
On Agar	12.9 ± 0.8	24.3 ± 3.9	33.5 ± 1.3	(189.5) ± 3.4	29.8 ± 1.4	242.6 ± 4.3	30.4 ± 1.2	120.8 ± 3.1

* Curvatures in coleoptiles shown as means of angles from 0°, not the mean positions.

3. WHEAT SEEDLINGS ON AGAR

A third method of seedling culture has been developed at the Ames Research Center by Dr. K. Yokoyama, the group leader in developing the wheat seedlings experiment. In collaboration with the co-experimenters, he has been germinating the same wheat seeds on a gel of 2% agar inside a moist chamber formed by a pair of Petri dishes. Growth takes place in a dark chamber at 77 ± 1° F.

A pre-soaked seed is set tightly in a small cavity in an agar plate, with the embryo clear of the surface but parallel to it. The chamber is supported so that the embryo is erect to gravity except in clinostat experiments for which the chamber is turned on the axis of the embryo at 1 revolution in 40 minutes. Other tests were made with the embryo either horizontal to gravity or inverted but Table V includes only data from tests that correspond to our 72-hour tests with seedlings in soil or seed holders of the preprototype hardware.

Since the seedling organs remain clear of the agar, the face view angles can be measured with a goniometer without opening the chamber. Side view angles can not be measured. Length of organs is measured in mm.

The data in Table V show that the seedlings grow on agar more rapidly than in our seed holders but neither experimental method supplies water through the endosperm at rates comparable with the rate of entry through roots in soil. On the whole the agar method is less reliable than the holder method in producing lateral roots at angles found in soil-grown plants. The tips of these roots are definitely lower than for seed holder plants, perhaps because of their greater length and weight of poorly supported tissues. However, these roots also have their tips much nearer the 180° line when grown on the horizontal clinostat, where the weight factor does not apply except possibly at the time of angle measurement.

Other differences between agar-grown seedlings and those in our seed holders include greater increases in length of coleoptile and lateral roots (but not primary root) for a clinostat plant. The cause of this difference in elongation rates by elimination of the effects of gravity is unknown.

Neither the length not the mean position of the primary root seems to be affected by the removal of gravity stimulation. The apparent increase in degrees for the primary root position of agar seedlings (see Table V, last line) is not informative since it does not take into account the actual mean curvature of the root from the 180° position. The number of displacements above and below this reference point has not been considered. The mean curvatures for the primary root have not yet been computed for primary roots on either seed holder or agar-grown seedlings but the data for curvatures in coleoptiles are recorded in Table V on the basis of corresponding mean variations from the 0° position. They show that the mean curvatures in coleoptiles increase on the clinostat for both seed holder and agar-grown seedlings, with the proportionately greater increment for agar-grown plants probably due to the greater length of the organ.

Certain other characteristics of growth of wheat seedlings on agar in comparison with growth in our seed holders are less interesting although they help to emphasize subtle differences in the microenvironment of the 2 culture methods by which roots can be caused to grow free of solid materials. The agar method, however, has no provisions for measurements of organ orientation in the third dimension. Since these measurements prove to be significant in comparisons between plants grown with and without the tropistic effects of gravity (see below and Tables III and IV), and because of the closer agreement in growth form with plants grown erect in soil, our seed holder method for the orbital experiment seems to be preferable as well as proven practical.

X. CONCLUSIONS FROM EXPERIMENTAL RESULTS

1. General

The reasonably good consistency in the orientation of seedling organs for wheat plants grown either erect or on clinostats, in seed holders of the form now accepted for our preprototype hardware, is very encouraging for good results from the orbital experiment. The variations in length of organs in a set of seedlings on one holder are not great enough to introduce unacceptable variations in the angles by which we shall measure orientation. The variations in data for these angles are also not so great as to interfere with the use of means from one set of seedlings for comparison with means from other experiments or with means in base line measurements.

Data for the orientation features of the 6 sets of seedlings that will grow in orbit will very probably be adequate for evaluation of the effects of zero gravity on the growth regulators within the seedling organs. At the same time the quantitative data for seedling growth in the absence of gravitational force will provide a unique check on the validity of the horizontal clinostat in simulating zero gravity in relation to geotropism.

2. Data from Coleoptiles

In spite of appreciable curvatures in some coleoptiles grown erect to gravity, the means of such displacements from the vertical are so small that the corresponding data for the face view and side view angles from the orbital experiment should provide useful criteria for the effects of zero gravity. The clinostat tests show larger means of curvatures that increase with the lengths of the coleoptiles.

3. Data from Primary Roots

The experimental data of our work give only partial support to Rufelt's idea (8) that the central root is plagiotropic rather than orthotropic. The means for its orientation angle in erect plants in our seed holders are only a few degrees from the vertical, as they are in soil and on agar (Table V) if measured in face view. The means of side view curvatures for erect plants (Tables III and IV) are seldom large or computed from bends in the same direction for a set of seedlings.

In the absence of gravitational stimulation, however, there is a tendency toward strong curvatures in one direction (toward the camera) as one would expect from a plagiotropic organ such as a branch (6) when the movement of the root's auxin toward the lower side (1) was prevented. There is no such tendency for hyponastic (or epinastic) curvature either above or below 180° in the face view (cf. record sheet data on p. 24). The "primary" root does show large curvatures in this face view plane when freed from the directive effects of gravity but the curvatures are random in size and direction. If this root is plagiotropic at all, the tendency is to grow outside the plane of the face of the embryo but in no one plane in the third dimension.

As a criterion of the formative effects of weightlessness in our orbital experiment, the side view angle of the primary root will be very important if the effects resemble those on the clinostat. The pattern of face view angles will probably be more significant than the mean for any one set of seedlings but the mean curvatures (not computed for Tables III to V) of the primary root in the plane of the lateral roots may turn out to be significant also.

4. Data from Lateral Roots.

Both face view and side view angles of the plagiotropic seminal roots can be used as criteria of orientation effects at zero gravity. As measured on the clinostat without directive effects of gravity, the angles have changed, in the plane of the two roots, from about 125° and 235° for erect seedlings to about 75° and 285°. In both cases the tips of the roots have been raised from the down position by 50°. This can be understood on an auxin control basis that corresponds to the condition demonstrated by Lyon (6) for lateral branches, since Boysen-Jensen (1) proved that auxin is unevenly distributed in horizontal roots. The changes in the side view angles of the same roots from about 20° for erect seedlings to angles about 6 times as great (see Tables III and IV) without the gravitational effect on the auxin does not mean that the lateral roots are plagiotropic also in the third dimension. The difference lies in the mixed + and - signs on the side view angles of both erect and clinostat seedlings, as illustrated in the record sheets on p. 24.

Until we experiment further with these lateral root curvatures, and particularly until we have the data from the orbital experiment, we cannot explain the behavior of these roots as viewed from the side of the embryo. The problem seems to be one of auxin origin and transport within the roots, as it probably is also for understanding the greater curvatures in the primary root in the absence of the effects of gravity. As criteria for the effects of weightlessness, however, the orientation angles for both face and side views of the lateral roots will be particularly valuable.

XI. PLANS FOR MORE EXPERIMENTAL STUDIES

We shall continue to seek improvement in techniques of loading and capping the seed holders. The 1965 seed crop will be tested as soon as it is available, with the hope that the vigor and percentage of germination will be very high. More data will be collected for the growth of rye, barley and perhaps oats for use in interpreting the orientation peculiarities of all these seedlings in terms of their growth regulators.

The question of orientation of the primary root of wheat, when grown either erect or on a clinostat, will be explored further by computations of the means of curvature from 180°. The results of this method for the coleoptile curvatures proved so instructive that we expect it to provide at least some information relative to Rufelt's plagiotropic theory (8). Our tridimensional method for measuring curvatures can probably be exploited to advantage in this field.

Until acceptable experiments can be carried out with applications of exogenous auxin to roots, the interpretations of growth curvatures will lack a sound foundation. With the wheat roots so slender and all roots so sensitive to contact with solid bodies, we expect to have major difficulties in supplying exogenous auxin to roots growing in our seed holders. We shall combine efforts in this area with attempts to grow uniform control seedlings by the use of the best materials that we can supply to the problem.

Our previous attempts to germinate Dicotyledonous seeds in special holders and moist chambers will be resumed as time permits.

We hope to perfect methods for growing roots large enough for use in quantitative experiments with auxin, much as we were able to do earlier with branches (6). The use of radioactive IAA-C^{14} on either epicotyls or thick roots still appears to be a promising line of approach to auxin physiology in seedlings. Some type of peas (Pisum) offer the best prospects for a test plant but bacterial infections are difficult to eliminate in our moist chambers. We must devise better methods of sterilization and culture before we can expect to grow good seedlings in the numbers required for quantitative data. We have some special apparatus for work with such seedlings and plan to use it soon.

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APPENDIX

COMPUTER PROGRAMS

1. Angles of a Coleoptile

```
  5  PRINT "ANGLE A, "ANGLE B"
 10  REM THIS PROGRAM MEASURES ANGULAR GROWTH OF GRASS
 20  REM COLEOPTILE WITH REFERENCE TO NORMAL ERECT POSITION
 30  LET C = 180/3.14159
 40  READ X, Y, Z
 50  LET B = ATN(Z/Y)*C
 60  LET A = ATN(X/Y)*C
 70  IF A >= 0 THEN 90
 80  LET A = 360 + A
 90  IF B >0 THEN 120
100  LET B = INT(10*B + .5)/10
110  GO TO 130
120  LET B = INT(10*B - .5)/10
130  LET A = INT(10*A + .5)/10
140  PRINT A, B
150  GO TO 40
900  DATA -6,27,22,0,16,4
999  END
```

(These data from p. 19; for angles see p. 23)

2. Angles of Roots

```
 10  REM THIS PROGRAM COMPUTES THE SOLID ANGLES OF THE THREE
 20  REM ROOTS OF THE PLANT GROWING UNDER VARIOUS CONDITIONS
 90  LET C = 180/3.14159
100  FOR I = 1 TO 3
110     READ X(I), Y(I), Z(I)
120  NEXT I
130  FOR I = 1 TO 3
140     IF I > 1 THEN 170
145     LET Y = Y(I)
150     PRINT "PR";
160     GO TO 210
170     IF I > 2 THEN 200
180     PRINT "LR";
190     GO TO 210
200     PRINT "RR";
210     LET Y = Y(I)
220     IF Y = 0 THEN 500
230     LET A = ATN( X(I) / Y(I) ) * C
240     LET B = ATN( Z(I) / Y(I) ) * C
250     IF Y > 0 THEN 280
260     LET A = A + 180
270     LET B = B + 180
280     IF A >= 0 THEN 300
```

(root program cont'd)

```
290      LET A = A + 360
300      IF B > 0 THEN 320
310      LET B = B + 360
320      LET B = B - 180
330      IF B >= 0 THEN 360
340      LET B = B - 0.05
350      GO TO 370
360      LET B = B + 0.05
370      LET A = A + 0.05
380      PRINT INT(10*A)/10; INT(10*B)/10
390   NEXT I
400   PRINT
410   GO TO 100
500   IF X(I) = 0 THEN 580
510   IF X(I) > 0 THEN 540
520   LET A = 270
530   GO TO 550
540   LET A = 90
550   IF Z(I) <> 0 THEN 590
560   PRINT A; "UNDEF"
570   GO TO 390
580   IF Z(I) = 0 THEN 660
590   IFZ(I) > 0 THEN 620
600   LET B = 90
610   GO TO 630
620   LET B = -90
630   IF X(I) <> 0 THEN 380
640   PRINT "UNDEF"; B
650   GO TO 390
660   PRINT "ATYPICAL CASE"
670   GO TO 390
900   DATA -26,-38,-65,-36,35,-29,36,10,-15
901   DATA 2,-62,-12,-40,-23,-11,45,-22,0
999   END
```

(these data from p. 19; for angles see p. 25)

NASA-Langley, 1967